Supporting Information

Biomolecular Ultrasound Imaging of Phagolysosomal Function

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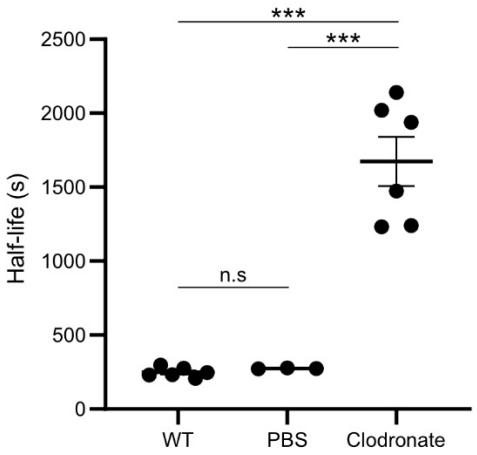

Figure S1: Circulation half-life of GVs, as measured by Doppler signal enhancement. Half-life was calculated as the time required for normalized signal enhancement to decline from its maximum at 1 to 0.5. Error bars represent ± SEM. N=6 (WT), 3 (PBS liposomes), 6 (clodronate liposomes). Welch's t-test (***: $p<0.001$; n.s: $p>0.05$).

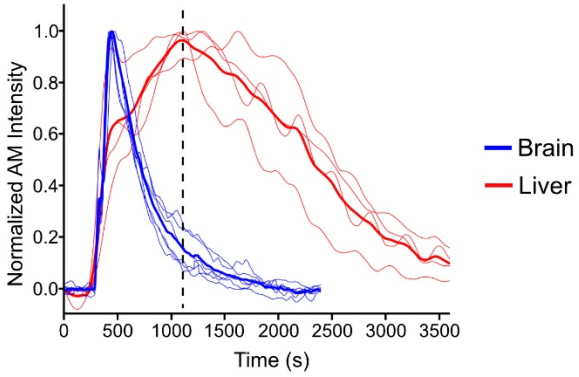

Figure S2: Time courses of ultrasound contrast in the brain (blue, n = 6) and liver (red, n = 4) of healthy C57BL/6 mice. Ultrasound contrast is essentially transferred from the brain to the liver, with liver contrast reaching its maximum (dashed line) after brain contrast dissipates. Thin lines, individual trials; thick lines, mean.

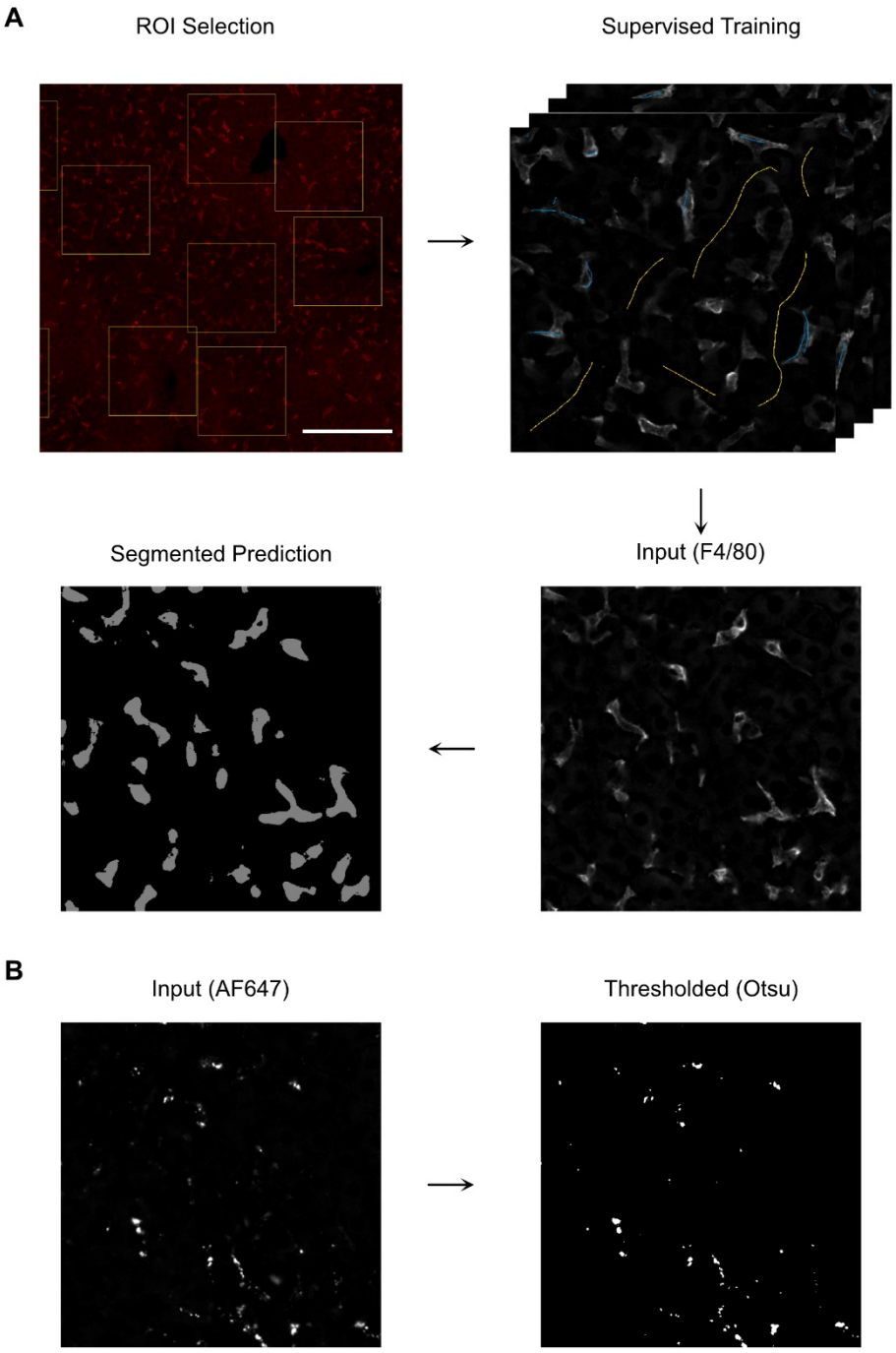

Figure S3: Segmentation protocol. **A)** Non-overlapping 500 px x 500 px (approx. 200 µm) ROIs were extracted from confocal micrographs of liver slices stained with anti-F4/80 (acquired with 20x objective, scale bar: 200 µm). We trained our Pixel Classification algorithm in ilastik by labeling background and macrophage regions on a subset of our images. Then, we segmented the remaining images by processing with the trained network. **B)** The corresponding images in the AF647 (GV) channel were automatically thresholded by Otsu's method, and colocalization was assessed in MATLAB.

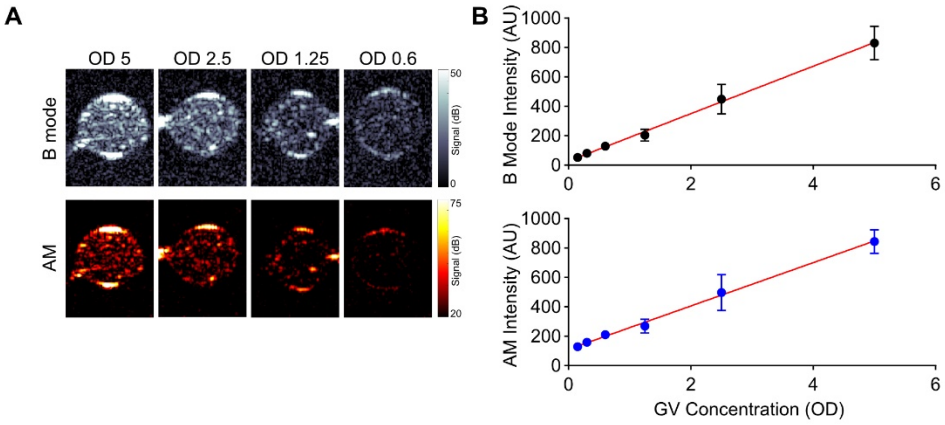

Figure S4: Ultrasound contrast is linear with respect to GV concentration. **A)** Representative B mode and AM images of non-linear GVs embedded in 1% agarose. Wells are approx. 2 mm in diameter. **B)** B mode (top) and AM (bottom) signal intensities. N = 12.

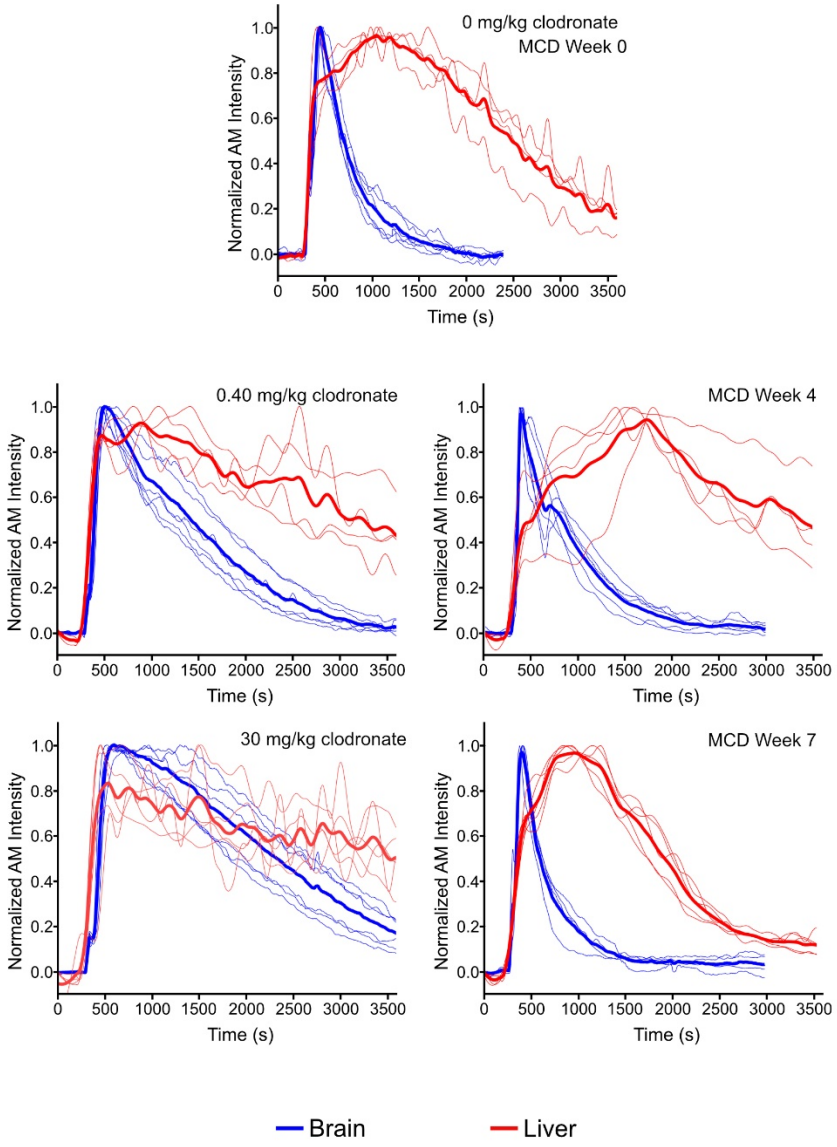

Figure S5: Ultrasound signal time courses used for estimating pharmacokinetic parameters. Brain, blue lines; liver, red lines; thin lines, individual trials; thick lines, mean.

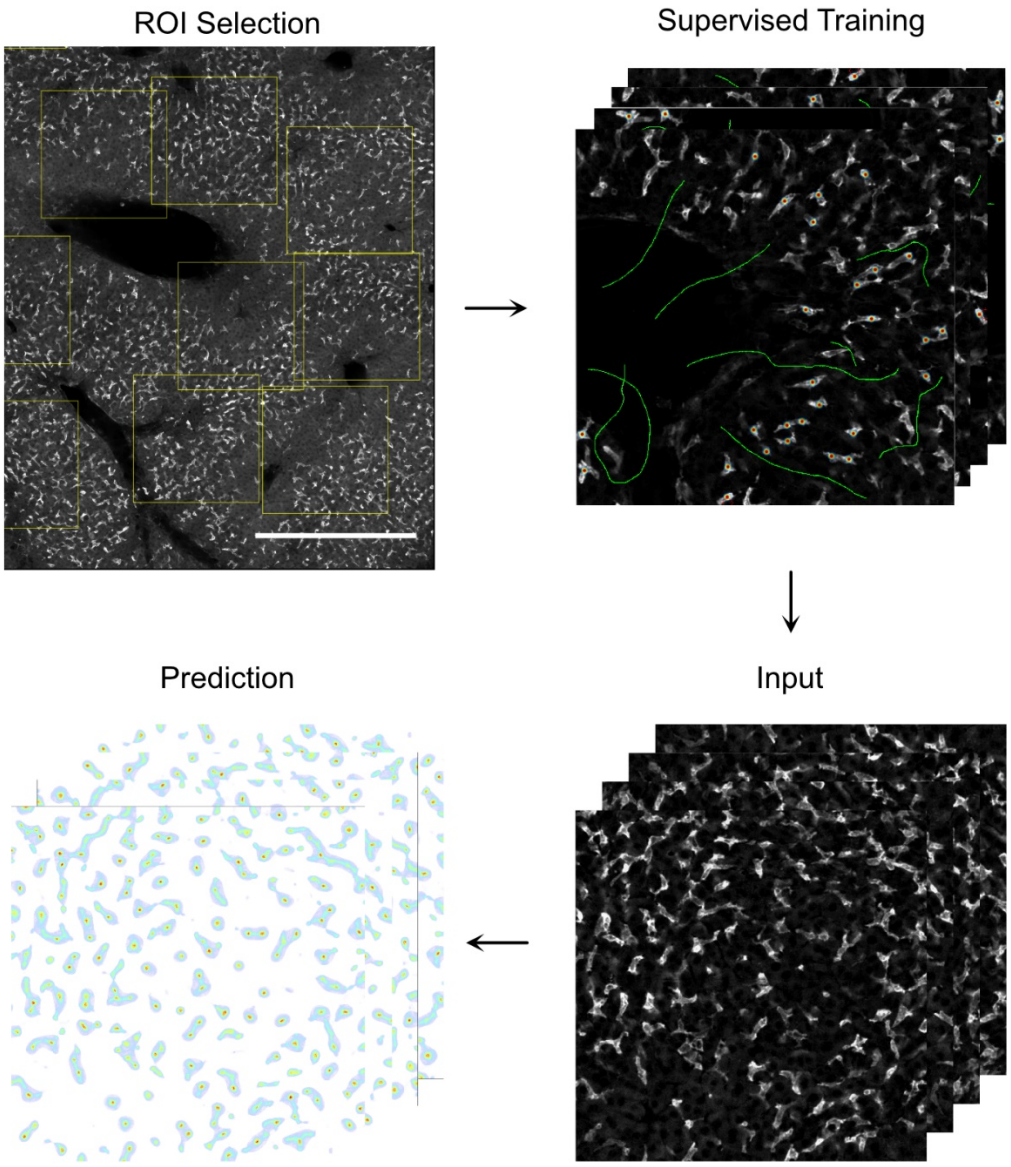

Figure S6: Processing for macrophage counting. Non-overlapping 500 px x 500 px (approx. 400 µm) ROIs were extracted from confocal micrographs of liver slices stained with anti-F4/80 (acquired with 10x objective, scale bar: 500 µm). Using the Density Counting workflow in ilastik, we annotated a subset of these images for background and cell bodies. Then, we processed the remaining images with our trained algorithm to predict macrophage density.

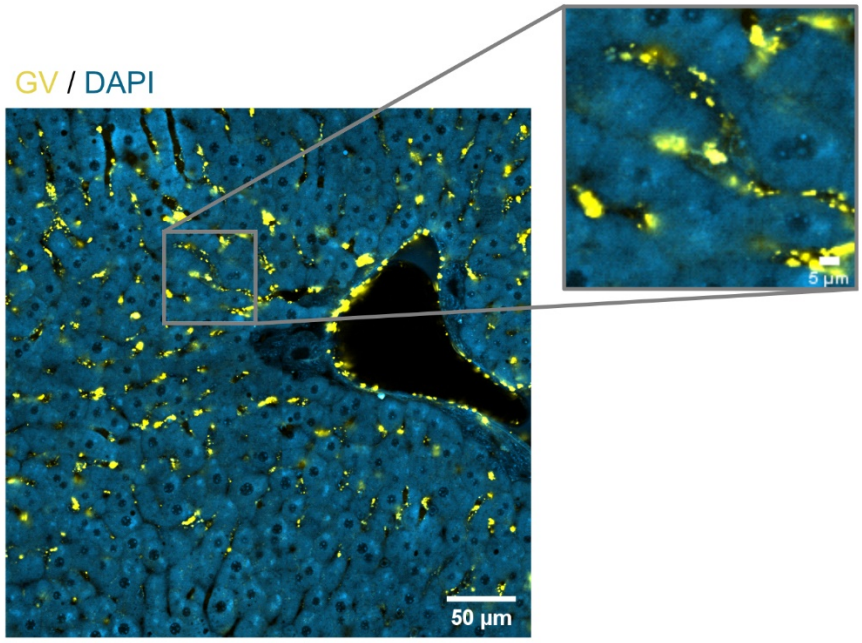

Figure S7: Confocal microscopy image of a liver section from a mouse treated with 30 mg/kg clodronate demonstrating localization of GVs to the sinusoidal periphery. Scale bars: 50 µm. Inset: 5 µm

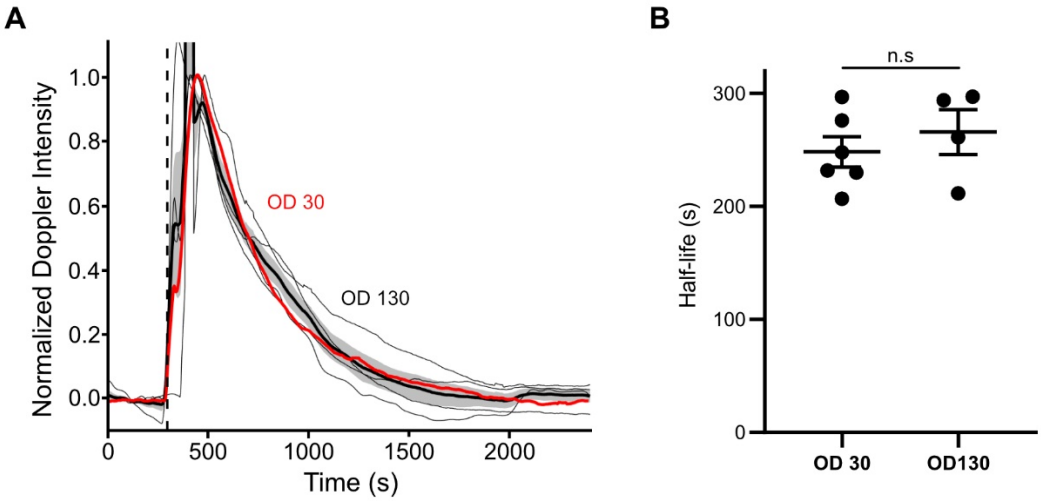

Figure S8: Hepatic clearance does not saturate under experimental conditions. **A)** Time course of ultrafast Doppler signal enhancement following IV injection of purified GVs at 300 s (dashed line). Individual traces, shown as thin lines, were normalized to their respective maxima. The thick line represents the mean of N = 4 biological replicates. Shaded area represents ± SEM. **B)** Half-lives of signal enhancement following IV injection of 100 µL GVs at OD30 or OD130. Welch's t-test (n.s: $p>0.05$).

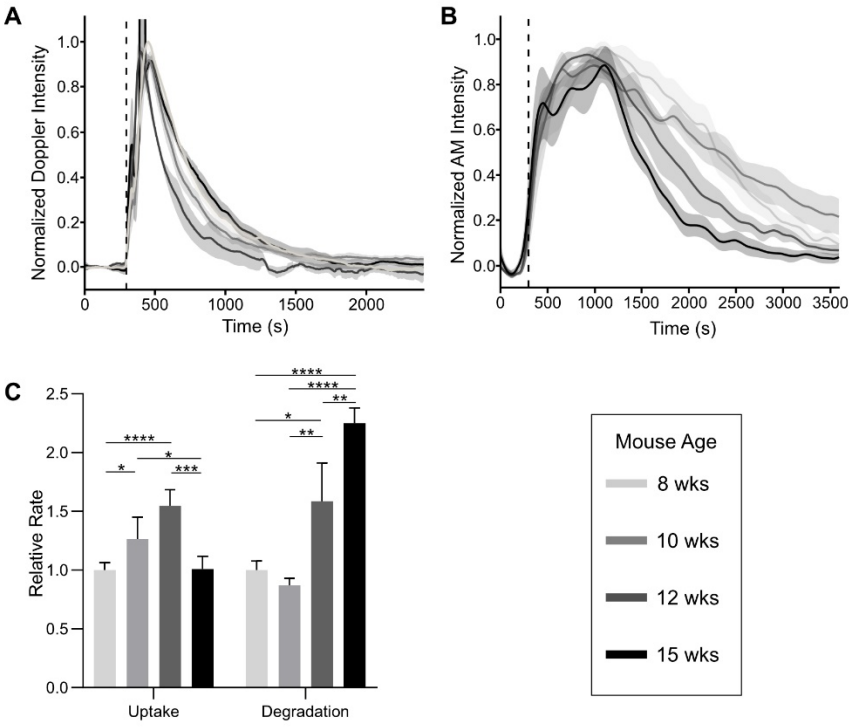

Figure S9: Hepatic macrophage activity changes with age. **A)** Time course of Doppler signal enhancement in mice of different ages following GV injection. Shaded areas represent ± SEM. N = 4-6 **B)** Time course of liver AM signal. Shaded areas represent ± SEM. N=3-5 **C)** Rates of GV uptake and degradation relative to those of 8 week old mice. Error bars represent ± SD. N=3-5. Welch's t-test(*:$p<0.05$; **:$p<0.01$).

Condition	Uptake Rate (min^{-1}, ± SD)	Degradation Rate (min^{-1}, ± SD)	k_c (± SD)
0 mg/kg clodronate	0.1667 ± 0.0107	0.0407 ± 0.0032	0.7572 ± 0.2328
0.40 mg/kg clodronate	0.0574 ± 0.0045	0.0299 ± 0.0053	0.6073 ± 0.2587
30 mg/kg clodronate	0.0299 ± 0.0010	0.0175 ± 0.0012	0.3843 ± 0.4507
MCD: 0 weeks	0.1667 ± 0.0107	0.0407 ± 0.0032	0.7572 ± 0.2328
MCD: 4 weeks	0.1087 ± 0.0108	0.0172 ± 0.0019	0.7268 ± 0.2899
MCD: 7 weeks	0.1818 ± 0.0068	0.0612 ± 0.0017	1.0000 ± 0.0000
Age: 8 weeks	0.1667 ± 0.0107	0.0407 ± 0.0032	0.7572 ± 0.2328
Age: 10 weeks	0.2108 ± 0.0310	0.0354 ± 0.0025	0.6478 ± 0.0859
Age: 12 weeks	0.2577 ± 0.0229	0.0645 ± 0.0132	0.9576 ± 0.0752
Age: 15 weeks	0.1681 ± 0.0181	0.0916 ± 0.0052	0.8879 ± 0.1546

Table S1: Constants derived from fitting pharmacokinetic model to ultrasound data. k_1, uptake rate; k_2, degradation rate; k_c, conversion constant.